Interactions of Cyclic AMP and Its Dibutyryl Analogue with a Lipid Layer in the Aqueous Mixtures of Monoolein Preparation and Dioleoyl Phosphatidylcholine as Probed by X-Ray Diffraction and Raman Spectroscopy

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Abstract. Interactions of adenosine 3':5'-cyclic monophosphate (cAMP) and N⁶,2'-O-dibutyryl-adenosine 3':5'-cyclic monophosphate (dbcAMP) with a lipid layer composed of monoolein-based preparation and dioleoyl phosphatidylcholine (DOPC) were investigated by small-angle X-ray diffraction (SAXD) and Raman spectroscopy. The reversed hexagonal (H_{II}) MO/DOPC/H₂O phase of 65:15:20 wt.% composition was selected as a reference system. SAXD revealed that entrapment (at the expense of water) of 3 wt.% cAMP into the reference system did not change the polymorphic form and structural parameters of the phase. The same content of dbcAMP induced the transition from the H_{II} phase to the reversed bicontinuous cubic phase of space group *Ia3d*. This transition is explained by the increase of lipid head-group area due to the penetration of the acylated adenine group of dbcAMP into the polar/apolar region of lipid layer. The conclusion is supported by Raman spectroscopy, showing the disruption/weakening of hydrogen bonding in the MO/DOPC-based matrix at the N1- and N3-sites of the dbcAMP adenine ring. As distinct from dbcAMP, cAMP remains mostly in the water channels of the H_{II} phase, although the phosphate residue of nucleotide interacts with the quaternary ammonium group of DOPC. Both nucleotides increase the population of *gauche* isomers in the DOPC choline group.

Key words: Cyclic AMP, dibutyryl cyclic AMP, dioleoyl phosphatidylcholine, liquid-crystalline phases, monoolein, Raman spectroscopy, X-ray diffraction

1. Introduction

Adenosine 3':5'-cyclic monophosphate (cAMP; Figure 1), one of the most important biological secondary messengers, plays a crucial role in most cellular events,

Figure 1. Molecular structures of adenosine 3':5'-cyclic monophosphate (cAMP), N⁶,2'-O-dibutyladenosine 3':5'-cyclic monophosphate (dbcAMP), 1-monooleoyl glycerol and dioleoyl phosphatidylcholine (DOPC).

and thus has received much attention of numerous investigators. A great number of synthetic analogues of cAMP have been synthesized in an effort to increase their biomembrane permeability by introducing lipophylic acyl groups into amino, sugar, and phosphate moieties. Among them, the most popular synthetic analogue of cAMP is N⁶,O^{2'}-dibutyryladenosine 3':5'-cyclic monophosphate (dbcAMP; Figure 1), which is widely applied as a molecular tool in biochemical studies, and has some therapeutic applications [1].

In an attempt to understand the factors which govern differences in the membrane permeability of these two cyclic nucleotides, we have investigated previously [2] the interactions of cAMP and dbcAMP with the lipid bilayer of monoolein (MO; as indicated below, 1-monooleoyl glycerol constitutes the greater part of MO, Figure1) by using small-angle X-ray diffraction (SAXD) and Raman spectroscopy methods. In that study, among several liquid-crystalline phases of aqueous MO [3], the reversed bicontinuous cubic phase of space group type *Pn3m* had been selected as a bilayer-based model system for some reasons. Firstly, the *Pn3m* cubic phase of MO can coexist with bulk water. Secondly, there is a good ground to claim that non-lamellar (e.g., bicontinuous cubic, reversed hexagonal) structures of lipids perform a number of biological roles [4–8].

Thus, in the MO-based model system, we have observed that, distinctly from cAMP, dbcAMP (at ca. 3 wt.% content) induces the intercubic *Pn3m* → *Ia3d* phase transition. Different effects of the MO phase matrix on the Raman shifts of the adenine and phosphate vibrational modes have enabled us to conclude that dbcAMP associates with MO bilayer, whereas the cAMP molecules for the most part remain in the water channels of the cubic phase [2].

The main motivation of the current study was to apply a lipid layer of more realistic composition by introducing phospholipids to the MO system. We focus especially on phosphatidylcholines, since they are among the most abundant phospholipids in membranes. It should be mentioned that the effect of cAMP (concentration range 10⁻⁶–10⁻² M) on phospholipids of human erythrocytes membranes has been studied previously by Raman spectroscopy [9]. It has been determined that cAMP slightly changes the packing of the aliphatic chains, somewhat decreases the all-trans portion of the chains, and, depending on the concentration, increases or decreases the mobility of the polar parts of membrane phospholipids. However, in that work, the possible types of intermolecular interactions have not been defined.

In the present study, the difference between the interactions of cAMP and dbcAMP with lipid bilayer again received primary consideration. However, 1,2-di(cis-9-octadecenoyl)-*sn*-glycero-3-phosphocholine (DOPC, Figure1) is chosen as an additional component of the MO-based bilayer. DOPC contains a zwitterionic head-group and the same hydrophobic oleoyl chain as MO. Cyclic nucleotides were incorporated into the MO/DOPC/H₂O mixture of 65:15:20 wt.% composition, which is considered as the reference system, in the quantity of about 3 wt.%. The reference and cAMP- or dbcAMP-containing samples are analyzed by employing

SAXD and Raman spectroscopy. The lipid-to-water content ratio was selected on the basis of phase diagram of the 1-monooleoylglycerol/DOPC/D₂O system presented by Gutman et al. [10] and Nilsson et al. [11]. The authors of those studies have shown that an isotropic liquid-crystalline sample of 65:15:20 wt.% composition belongs to the cubic phase of body-centered *Ia3d* or *Im3m* space group.

In more recent investigations of the aqueous 1-monooleoylglycerol/DOPC mixture [12, 13] it has been demonstrated that the fully hydrated (≥ 60 wt.% of water) *Pn3m* phase of 1-monooleoylglycerol can accommodate up to 25 mol% of DOPC. However, the spectroscopic investigation of these MO/DOPC samples is complicated since two separate phases, aqueous and lipidic, coexist at the excess water condition. For this reason, in the present study, the fully hydrated MO/DOPC mixture was ruled out in favour of the above-mentioned MO/DOPC/H₂O mixture of 65:15:20 wt.% composition.

2. Materials and Methods

The mixture of mono- and diglycerides (about 25:1 by weight), denoted as mono-olein (MO), was kindly provided by Danisco Ingredients (Brabrand, Denmark) with the following acyl chain composition (batch TS-ED 173): 90 wt.% oleic acid, 5 wt.% linoleic acid, 2.7 wt.% stearic acid, 1 wt.% palmitic acid, 0.3 wt.% linolenic acid, and 1 wt.% other fatty acids. The same batch of MO was used in all experiments. 1,2-Di(cis-9-octadecenoyl)-*sn*-glycero-3-phosphocholine (DOPC; purity ca. 99%, from Sigma), sodium salt of adenosine 3':5'-cyclic monophosphate (cAMP; purity ca. 98%, from Reanal, Hungary), sodium salt of N⁶,2'-O-dibutyryl-adenosine 3':5'-cyclic monophosphate (dbcAMP; purity ca. 97%, from Sigma) were used as received. The water was distilled and passed through a Milli-Q water purification system (Millipore S.A., Molsheim, France).

Samples were prepared by weighing of ca. 300 mg of MO/DOPC mixture (13:3 by weight) in the 8 mm (i.d.) glass ampoules (Duran[®], Schott Glaswerke, Mainz, Germany), melting MO at 40 °C, adding appropriate volumes of water or of the cAMP or dbcAMP solutions in water, and gently mixing the content. The ampoules were flame-sealed, and the samples were then centrifuged several times at 3000 × *g* and 25 °C before storing them in the dark at 20–23 °C for at least 2 weeks to ensure equilibration.

Small-angle X-ray diffraction (SAXD) studies were performed at 20 °C using a Kratky compact small angle system equipped with a position sensitive detector OED 50M (MBraun, Graz, Austria) containing 1024 channels of width 53.1 μm. Cu K_α nickel-filtered radiation ($\lambda = 1.542 \text{ \AA}$) was provided by a Seifert ID 3000 X-ray generator (Rich. Seifert & Co, Ahrensburg, Germany), operating at 50 kV and 40 mA. Diffraction data were collected for 2–3 h at the sample-to-detector distance of 277 mm. Temperature control within 0.1 °C was achieved by using a Peltier element.

Raman spectra were recorded by a DFS-24 spectrometer (Leningradskoje Optiko-Mekhanicheskoye Ob'yedineniye, St. Petersburg, Russian Federation) with samples in the above-mentioned glass ampoules using a 90° scattering geometry. An argon ion laser LGN-503 (Lvov, Ukraine) was used for excitation of the samples at the 514.5 nm line. The laser plasma lines were attenuated by means of a holographic laser bandpass filter (Kaiser Optical Systems, Inc., USA). Spectra were recorded at 2-cm^{-1} resolution and with a laser power of ca. 50 mW. The total integration time was 12–16 s per point, resulting from the summation of 3–4 spectra. The Raman scattering light was analyzed by a $f/5.3$ double monochromator with 1200 lines/mm gratings, and detected by a water cooled (to ca. 10°C) photomultiplier and a photon counting system. Elastic scattering was eliminated with a holographic SuperNotch[®] filter (Kaiser Optical Systems, Inc., USA). Spectroscopic experiments were performed at $20\text{--}23^\circ\text{C}$. The spectral frequencies were calibrated with the argon-ion laser plasma lines, and are believed to be accurate to $\pm 1\text{ cm}^{-1}$. The overlapping Raman bands were deconvoluted into the sum of Gaussian and Lorentzian shapes. Positions, bandwidths and amplitudes were varied until good agreement was achieved between actual and simulated spectra (correlation coefficients r were no less than 0.999).

3. Results and Discussion

3.1. SMALL-ANGLE X-RAY DIFFRACTION STUDY

Small-angle X-ray diffraction (SAXD) data of the samples studied are presented in Figure 2.

As distinct from the 1-monooleoylglycerol/DOPC/ D_2O system of 65:15:20 wt.% composition [10, 11], the diffraction patterns obtained for the reference (MO/DOPC/ H_2O of 65:15:20 wt.% composition) and cAMP-containing samples are characteristic of a two-dimensional hexagonal lattice with Bragg reflections having a spacing ratio 1 (10): $\sqrt{3}$ (11): 2 (20) (the Miller indices are given in the brackets). As shown in Figure 2, two corresponding plots of d_{hk} vs. $(h^2 + k^2 + hk)^{-1/2}$ give straight lines passing through the origin and giving hexagonal lattice constants (a_0 ; cylinder center-center distance) of 57.4 ± 0.2 (value $\pm$ S.E. of the linear fit; $r^2 = 0.9999$) and $55.8 \pm 0.5 \text{ \AA}$ ($r^2 = 0.9997$), respectively. In contrast, six Bragg peaks appear in the SAXD pattern of the dbcAMP-containing sample with spacings having a ratio $\sqrt{6}$ (211): $\sqrt{8}$ (220): $\sqrt{14}$ (321): $\sqrt{16}$ (400): $\sqrt{20}$ (420): $\sqrt{22}$ (332), consistent with the body-centered cubic lattice of space group $Ia3d$. A plot of d_{hkl} vs. $(h^2 + k^2 + l^2)^{-1/2}$ (Figure 2) results in the unit cell axis a_0 of $110.2 \pm 0.2 \text{ \AA}$ ($r^2 = 0.9999$).

As already mentioned above, our SAXD result from the reference MO/DOPC/ H_2O system differs from that reported on the 1-monooleoylglycerol/DOPC/ D_2O mixture of the same composition. In the latter system, Gutman et al. [10] and Nilsson et al. [11] have detected formation of the reversed hexagonal phase (H_{II}) only at low heavy water content ($< 10\text{ wt.}\%$). At higher D_2O content, including

Figure 2. Plot of d_{hkl} vs. $(h^2 + k^2 + l^2)^{-1/2}$ of the reflections obtained for the MO/DOPC/H₂O/dbcAMP sample of 65:15:17:3 wt.% composition (cubic phase of space group *Ia3d*), and plots of d_{hk} vs. $(h^2 + k^2 + hk)^{-1/2}$ of the reflections obtained for the reference MO/DOPC/H₂O sample of 65:15:20 wt.% composition and MO/DOPC/H₂O/cAMP sample of 65:15:17:3 wt.% composition (reversed hexagonal phases, H_{II}).

the sample of 65:15:20 wt.% composition, a large area of the bicontinuous cubic phase has been determined.

By analogy to the 1-monooleoylglycerol/DOPC/D₂O system, a type II structure (reversed) is assigned to the hexagonal phase of the reference MO/DOPC/H₂O sample (Figure 2). We believe that the cubic/H_{II} boundary of our sample is shifted towards higher water content because of admixed diglycerides and polyunsaturated monoglycerides in the MO preparation (recall that monooleoylglycerol comprises

only ca. 86 wt.% of the preparation), which promote the formation of reversed mesophases [14–16].

However, the most interesting experimental fact is that entrapment of 3 wt.% dbcAMP into the reference lipidic system induces the $H_{II} \rightarrow Ia3d$ phase transition, whereas the same quantity of cAMP leaves the mesophase structure and its parameters practically unchanged. As mentioned in the introduction, these nucleotides affect the cubic $Pn3m$ phase of aqueous MO in an analogous fashion [2].

The lipid aggregates and their transformations are often analyzed by using a dimensionless lipid shape parameter v/al , where v is the hydrocarbon chain volume, a is the head-group area at the hydrocarbon/water interface, and l is the maximum effective length of the acyl chain [17]. By applying this concept to the $H_{II} \rightarrow Ia3d$ phase transition in our system, it appears that dbcAMP decreases v/al since, at the same hydration, the molecular shape parameter for the H_{II} phase invariably exceeds the value for the reversed bicontinuous cubic phase [18]. One should anticipate that this effect is determined by the molecular interactions between dbcAMP and the components of MO/DOPC layer. Since Raman spectroscopy provides valuable information on the molecular interactions and dynamics, this technique was selected for further investigation of the intermolecular features in our systems.

3.2. RAMAN SPECTROSCOPY OF THE AQUEOUS MO/DOPC

LIQUID-CRYSTALLINE PHASES WITH ENTRAPPED CYCLIC NUCLEOTIDES

Raman spectra of the above-mentioned pseudoternary and pseudoquaternary liquid-crystalline phases over the wavenumber range of 600–1800 cm^{-1} are presented in Figures 3 and 4.

Since the MO/DOPC-based phases contain low amounts of cAMP or dbcAMP, only a few bands associated with these cyclic nucleotides can be observed directly in the Raman spectra. These are: (i) the bands at 1509 and 1580 cm^{-1} , and a shoulder at around 1338 cm^{-1} in the spectrum of the cAMP-containing H_{II} phase (Figure 3, spectrum 2), and (ii) the features at 1168, 1355 and 1592 cm^{-1} in the spectrum of the dbcAMP-containing cubic phase (Figure 4, spectrum 2). Other bands of nucleotides are overlapped by strong features of MO and/or DOPC. However, using the digital difference spectra shown by traces 4 in Figures 3 and 4 (the spectrum of the nucleotide-containing sample minus the spectrum of the reference liquid-crystalline phase, satisfying a criterion of zero intensity of the C=C stretching vibration band at 1655 cm^{-1}), we were able to retrieve a number of bands that are attributable to the entrapped cyclic nucleotides. As it has been shown previously [2], the comparison of these difference spectra with the spectra of nucleotides in water (traces 3 in Figures 3 and 4) is particularly useful for detecting the interactions of nucleotides with model lipid membrane.

Traces 3 and 4 in Figure 3 show that the entrapment of cAMP in the H_{II} phase of aqueous MO/DOPC mixture leaves the frequencies of the most cAMP vibrational bands unchanged, with two exceptions. Thus, the bands corresponding to the sym-

Figure 3. Raman spectra of: (1) the hexagonal H_{II} MO/DOPC/ H_2O phase of 65:15:20 wt.% composition; (2) the hexagonal H_{II} MO/DOPC/ H_2O /cAMP phase of 65:15:17:3 wt.% composition; (3) the 0.6 M cAMP solution in H_2O ; and (4) the digital difference spectrum (2)-(1) (spectrum of the cAMP-containing H_{II} phase minus spectrum of the reference H_{II} MO/DOPC/ H_2O phase, satisfying a criterion of zero intensity of the 1655-cm^{-1} MO/DOPC peak). Excitation, 514.5 nm. Integration time, 4 s. Three scans were co-added for every spectrum. For clarity, spectra were shifted and multiplied.

Figure 4. Raman spectra of: (1) the reversed hexagonal H_{II} MO/DOPC/ H_2O phase of 65:15:20 wt.% composition; (2) the cubic $Ia3d$ MO/DOPC/ H_2O /dbcAMP phase of 65:15:17:3 wt.% composition; (3) the 0.3 M dbcAMP solution in H_2O ; and (4) the digital difference spectrum (2)-(1) (spectrum of the dbcAMP-containing cubic phase minus spectrum of the reference H_{II} MO/DOPC/ H_2O phase, satisfying a criterion of zero intensity of the 1655 cm^{-1} MO/DOPC peak). Excitation, 514.5 nm. Integration time, 4 s. Three scans were co-added for spectra (1) and (2). Four scans were co-added for spectrum (3). For clarity, spectra were shifted and multiplied.

Table I. Positions and shifts (values in the brackets; relative to the peaks in the aqueous nucleotide solutions) of the environment-sensitive Raman bands ($\nu_s(\text{PO}_2^-)$ – symmetric vibration of PO_2^- group; Pyr – vibration localized mainly on the six-membered pyrimidine ring) of the cyclic nucleotides upon entrapment into the MO- (data from [2]) and MO/DOPC-based matrices (this work)

System	Raman mode (cm^{-1})		
	$\nu_s(\text{PO}_2^-)$	Pyr	Pyr
cAMP in water	1088	1305	1339
cAMP in the $Pn3m$ cubic phase of MO	1094 (+6)	1310 (+5)	1338 (–1)
cAMP in the H_{II} phase of MO/DOPC	1084 (–4)	1301 (–4)	1338 (–1)
DbcAMP in water	1093	1294	1359
DbcAMP in the $Ia3d$ cubic phase of MO	1096 (+3)	1287 (–7)	1356 (–3)
DbcAMP in the $Ia3d$ cubic phase of MO/DOPC	?	1288 (–6)	1355 (–4)

metric vibration of PO_2^- group ($\nu_s(\text{PO}_2^-)$) at 1088 cm^{-1} and vibration localized mainly on the six-membered pyrimidine ring (Pyr) at 1305 cm^{-1} (assignments of the modes see in [2]) undergo a downshift by ca. 4 cm^{-1} . Inspection of traces 3 and 4 in Figure 4 reveals that, in the dbcAMP-containing cubic phase, a downshift is observed for the nucleotide bands at 1294 and 1359 cm^{-1} (both Pyr). The $\nu_s(\text{PO}_2^-)$ band in the difference spectrum of the entrapped dbcAMP is not clearly expressed and seems to be not shifted.

Comparison of the above Raman data with the results of our previous spectroscopic work on the MO/ H_2O /cAMP(dbcAMP) systems [2] reveals an interesting feature. In the aqueous nucleotide solutions, incorporation of DOPC into the MO-based matrix shifts the above-mentioned $\nu_s(\text{PO}_2^-)$ and Pyr bands of the entrapped cAMP in the *opposite direction* when compared to the pure MO system, whereas the two Pyr bands of the entrapped dbcAMP shift in the *same direction* and practically by the same values in both model membrane systems (Table I). The latter fact implies that dbcAMP interacts with the MO- and MO/DOPC-based layers in much the same way, and possible electrostatic attraction due to the presence of the DOPC choline group is not significant in our system. By contrast, indications are that the shift of cAMP's $\nu_s(\text{PO}_2^-)$ mode in the H_{II} phase of aqueous MO/DOPC mixture (see Table I) is related to the electrostatic interaction. For example, the studies of dialkylphosphates have demonstrated a decrease of the $\nu_s(\text{PO}_2^-)$ mode frequency upon formation of the $\text{PO}_2^- \cdots \text{HOH}$ hydrogen bond [19, 20], and the effect becomes even more pronounced (further $3\text{--}5\text{ cm}^{-1}$ downshift) by the additional interaction with cations [21].

From the position of cAMP's $\nu_s(\text{PO}_2^-)$ band in spectrum 3 of Figure 3, it follows that the phosphate group of this nucleotide in aqueous solution is hydrogen bonded to water ([2] for more details). Therefore, the observed further downshift of this band in the MO/DOPC-based H_{II} phase (Table I) most prob-

ably points to the interaction between the strongly basic quaternary ammonium group of DOPC choline and the phosphate group of cAMP. We cannot rule out the formation of a complex, in which two charged groups are separated by water – $(\text{CH}_3)_3\text{N}^+ \cdots \text{O}(\text{H})-\text{H} \cdots \text{O}_2\text{P}^-$.

Some so-called ‘marker’ bands, which are sensitive to the intermolecular interactions at a particular site of the adenine ring, are especially useful for the interpretation of the frequency shifts observed in the difference spectra of cyclic nucleotides. Intensive cAMP bands at 1305 and 1339 cm^{-1} in aqueous solution (Figure 3, spectrum 3), arising due to the vibrations localized mainly on the Pyr ring (corresponding dbcAMP modes are at 1294 and 1359 cm^{-1} ; Figure 4, spectrum 3), have been shown to be sensitive to the formation/disruption of hydrogen bonds at the N1- and N3-sites (see Figure 1) of adenine, respectively [22]. Following the interpretations [22], the downshift ($\sim 3 \text{ cm}^{-1}$; Table I) of the 1305- cm^{-1} cAMP band when going from aqueous solution to the MO/DOPC-based matrix is attributable to the weakening of hydrogen bonding at the N1-site of Pyr, whereas the entrapped dbcAMP presumably undergoes disruption and/or weakening of hydrogen bonds at both sites of protonation. The cAMP mode at 1509 cm^{-1} (Figure 3, spectrum 3), associated with the vibration localized mainly on the five-membered imidazole (Im) ring (corresponding mode of dbcAMP is at 1493 cm^{-1} ; Figure 4, spectrum 3), is the marker band of hydrogen bonding and complexation at the adenine N7-site [23, 24]. The intensity of this band, relative to the peak at 1482 cm^{-1} (Pyr + Im vibrational mode), has been found to decrease with increasing strength of the hydrogen bonding interaction at the C6-NH₂ site [25]. Both bands retain their frequencies and intensity ratio when cAMP is incorporated into the MO/DOPC-based lipid matrix (Figure 3, spectra 3 and 4), indicating that environment of the C6-NH₂ group of adenine in the lipid matrix is the same as in the aqueous solution. However these two marker bands are not well resolved in the case of dbcAMP and were assigned to one broad peak at 1493 cm^{-1} in the spectrum of the aqueous solution of cyclic nucleotide [2]. In the difference spectrum of the entrapped dbcAMP (Figure 4, spectrum 4) this band is considerably weakened. Further systematic Raman studies of dbcAMP dissolved in different solvents are required to establish the reliable marker bands sensitive to H-bonding interactions at the C6-NH- site.

Another interesting feature of the difference spectrum of the entrapped dbcAMP (Figure 4, spectrum 4) is a clearly resolved band at 716 cm^{-1} , which is absent in the spectrum of the aqueous solution (Figure 4, spectrum 3). In the difference spectrum of the entrapped cAMP (Figure 3, spectrum 4), a prominent in-plane vibrational mode of Pyr + Im is observed at 725 cm^{-1} that is asymmetric and of somewhat higher intensity relative to the spectrum in the aqueous solution of cAMP (Figure 3, spectrum 3). Deconvolution of this feature in the difference spectrum also reveals the presence of weak band at 716 cm^{-1} (data not shown). The latter band is representative of C-N symmetric stretching mode of the choline segment -O-CH₂-CH₂-N⁺(CH₃)₃ in a *gauche* conformation [26]. Therefore, there are grounds to

believe that cyclic nucleotides induce the conformational rearrangement of DOPC head-group by increasing the population of *gauche* isomers.

The influence of the studied nucleotides on the apolar hydrophobic region of the MO/DOPC-based layer might be analyzed by their effects on the parameters of the definite lipid bands. To this end, the difference spectra were obtained by the subtraction of the nucleotide aqueous solution spectrum from the spectrum of the corresponding pseudoquaternary liquid-crystalline phase. The subtraction was performed to satisfy a criterion of zero intensity of the 1580 and 1590 cm^{-1} bands for cAMP and dbcAMP, respectively. We analyzed the obtained difference spectra in the region of 1200–1350 cm^{-1} , containing in-plane $=\text{C-H}$ deformation, $\delta(=\text{CH})$, methylene twisting, $\delta_t(\text{CH}_2)$, and methylene deformation, $\delta(\text{CH}_2)$, bands. It has been demonstrated previously that the $\delta_t(\text{CH}_2)$ mode becomes broader with an increase in the conformational disorder in the hydrocarbon chains of lipids [27, 28], while the $\delta(\text{CH}_2)$ mode is sensitive to the interchain interactions [28]. However, no reliable differences between the reference and nucleotide containing samples in the regions of these two bands were observed. The same is true for the C-H stretching vibration region (2800–3000 cm^{-1}), which is more sensitive to the conformational rearrangements and lateral interaction of the hydrocarbon chains of lipids. Therefore, all these difference spectra are not shown and discussed in greater detail.

4. Conclusions

Interactions of two cyclic nucleotides, adenosine 3':5'-cyclic monophosphate (cAMP) and N⁶,2'-O-dibutyryl-adenosine 3':5'-cyclic monophosphate (dbcAMP), with a lipid layer composed of monoolein (MO) and dioleoyl phosphatidylcholine (DOPC) were studied by small-angle X-ray diffraction (SAXD) and Raman spectroscopy. The SAXD data revealed that the reversed hexagonal (H_{II}) MO/DOPC/ H_2O phase of 65:15:20 wt.% composition, which was selected as a reference system, retained its crystallographic structure and did not change the lattice parameters by the incorporation (at the expense of water) of about 3 wt.% of cAMP, whereas the same amount of dbcAMP induced the $H_{II} \rightarrow$ cubic $Ia3d$ phase transition. Based on the concept of a dimensionless lipid shape parameter (v/al), the latter transition should be accompanied by the decrease of v/al . It is conceivable that the partitioning of the dbcAMP's acylated adenine group into the MO/DOPC layer could lead to this effect by, for example, increasing the interfacial area of the lipid head-group (a). Indeed, this conclusion was confirmed by the Raman spectroscopic data for the MO/DOPC/ H_2O /dbcAMP system, which indicated that the hydrogen bonding at the N1- and N3-sites of the dbcAMP adenine ring was disrupted/weakened. However, when compared to the MO/DOPC/ H_2O system, the spectroscopic parameters of the lipid hydrocarbon chains in the MO/DOPC/ H_2O /dbcAMP sample remained unaltered. This suggests that the penetration of dbcAMP into the lipid layer is apparently not deep. According to the present SAXD and Raman spectroscopic data,

it seems that cAMP remains in the water channels of the H_{II} MO/DOPC/ H_2O /cAMP phase, although it interacts with the quaternary ammonium group of the DOPC choline residue through the phosphate moiety. Moreover, as evident from our Raman spectroscopy experiments, both cyclic nucleotides exhibit the capacity to increase the population of *gauche* isomers in the DOPC choline group.

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